

Structure of a model salt bridge in solution investigated with 2D-IR spectroscopy

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Abstract

Salt bridges are known to be important for the stability of protein conformation, but up to now it has been difficult to study their geometry in solution. Here we characterize the spatial structure of a model salt bridge between guanidinium (Gdm^+) and acetate (Ac^-) using two-dimensional vibrational (2D-IR) spectroscopy. We find that as a result of salt bridging the infrared response of Gdm^+ and Ac^- change significantly, and in the 2D-IR spectrum, salt bridging of the molecules appears as cross peaks. From the 2D-IR spectrum we determine the relative orientation of the transition-dipole moments of the vibrational modes involved in the salt bridge, as well as the coupling between them. In this manner we reconstruct the geometry of the solvated salt bridge.

The stabilization of molecular conformations by the attractive interaction between oppositely charged ions (salt bridges) is of great relevance in many areas of science. In particular, biological systems such as proteins, often contain salt bridges between ionic side chains that determine their structure [1] and function. [2] It is therefore fundamental to characterize the solvated structure of salt-bridged ion pairs, but this is unfortunately not possible with conventional methods like NMR. In this communication we report the study of a biologically-relevant ion pair, that formed by guanidinium (Gdm^+) and acetate (Ac^-), using two-dimensional infrared (2D-IR) spectroscopy. This ion pair is a model for salt bridges between an arginine and a carboxylate side group (from glutamate or aspartate), which occur commonly in proteins. [3] The molecular structure of this ion pair is shown in Fig. 1A. Isolated Gdm^+ has D_3 symmetry and a degenerate mode at 1600 cm^{-1} due to a combined CN_3 antisymmetric stretch and NH_2 scissors motion. [4] This degeneracy is also observed in aqueous solution, but it is broken in viscous solvents. [5] When dissolving deuterated Gdm^+ (guanidine·DCl, >98% purity) in deuterated dimethylsulfoxide (DMSO), we observe a similar splitting between the frequencies of the two CN_3D_6^+ modes, as can be seen in Fig. 1B. In the following, we will refer to the high- and low-frequency CN_3D_6^+ of Gdm^+ as the Gdm_{HF}^+ and Gdm_{LF}^+ modes, respectively.

Interestingly, when an equimolar amount of Ac^- ions is added to the solution (guanidine acetate salt, >98% purity), this splitting becomes larger. It is known that Gdm^+ and Ac^- have a strong binding affinity in DMSO, forming more than 98% dimers at the concentration used in our experiments. [6] This suggests that the larger splitting between the Gdm^+ modes is due to an interaction with the Ac^- ion. Moreover, Ac^- (tetrabutylammonium acetate, >97% purity) has an absorption band at 1580 cm^{-1} in DMSO (shown in Fig. 1B) due to the COO^- antisymmetric stretch mode. This mode red-shifts after dimerization with Gdm^+ . The change in the infrared response of both the Gdm^+ and the Ac^- ions upon dimerization strongly suggests that there is

a coupling between the vibrational modes of these two molecules.

The 2D-IR spectrum of $\text{Gdm}^+ \cdots \text{Ac}^-$ confirms unambiguously that the Gdm_{HF}^+ and Gdm_{LF}^+ modes are both coupled to the COO^- stretch mode of Ac^- . We use a femtosecond pump-probe setup that has been described elsewhere. [7] The resulting spectra are shown in Figs. 2B and C for parallel and perpendicular polarization of the pump and probe pulses, respectively. The non-zero off-diagonal response in the 2D-IR spectrum indicates that there is a coupling between the two CN_3D_6^+ modes of Gdm^+ and, more importantly, between each of them and the COO^- stretch mode of Ac^- . These cross peaks can be seen better in slices along both the pump and probe axes of the 2D-IR spectra. Fig. 3A shows a cross section for $\nu_{\text{pump}} = \nu_{\text{COO}^-}$ for parallel and perpendicular polarization of the pump and probe pulses. In the cross section, the cross peaks between the COO^- stretch mode and each of the two CN_3D_6^+ modes are clearly visible. Note that the diagonal response of the COO^- stretch

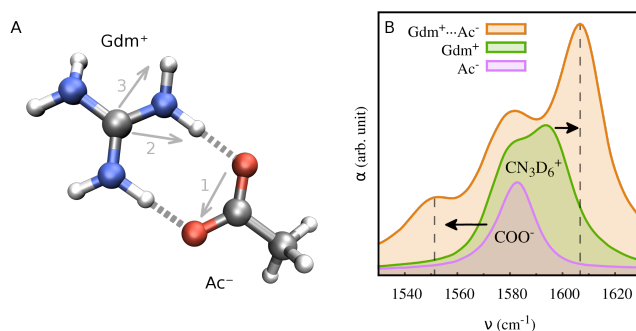


FIG. 1. (A) Molecular structure of the $\text{Gdm}^+ \cdots \text{Ac}^-$ dimer obtained using *ab initio* methods. The corresponding transition-dipole moments of the COO^- stretch mode (1) and of the CN_3D_6^+ low and high frequency modes (2 and 3, respectively) are indicated by arrows. (B) Infrared absorption spectrum of $\text{Gdm}^+ \cdots \text{Ac}^-$, Gdm^+ and Ac^- in DMSO (400 mM, solvent subtracted). Shifts of the COO^- and high-frequency CN_3D_6^+ bands are indicated by arrows.

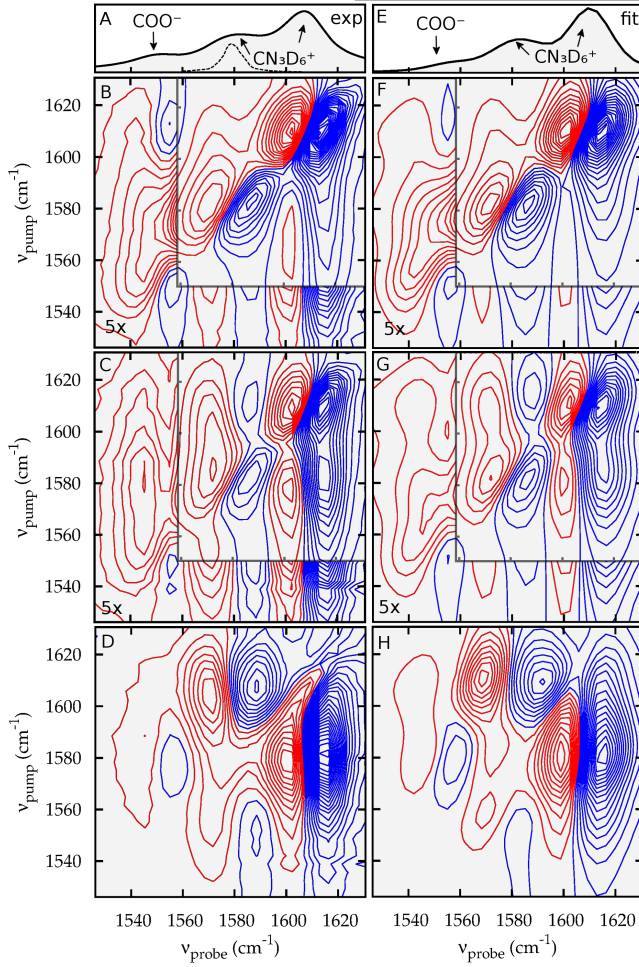


FIG. 2. (A) Infrared absorption spectrum of $\text{Gdm}^+ \cdots \text{Ac}^-$. The dashed line indicates a representative pump-pulse spectrum. (B) and (C) 2D-IR spectra of $\text{Gdm}^+ \cdots \text{Ac}^-$ for parallel and perpendicular polarization of the pump and probe pulses, respectively (pump-probe delay of 2 ps). Blue indicates negative absorption change, red positive absorption change. The contour intervals are 1 mOD (parallel) and 0.5 mOD (perpendicular), and the left and bottom parts are scaled by a factor of five, as indicated. (D) Difference 2D-IR spectrum between parallel and three times perpendicular polarization of the pump and probe pulses, the contour intervals are 0.5 mOD. (E) Calculated absorption spectrum with parameters obtained from a fit to the 2D-IR spectrum. (F) and (G) Fitted 2D-IR spectra for parallel and perpendicular polarization of the pump and probe pulses, respectively. The contour intervals are the same as in (B) and (C). (H) Calculated difference 2D-IR spectrum, with the same contour intervals as in (D).

mode has a smaller magnitude than these cross peaks because of its smaller absorption cross section, see Fig. 1. Fig. 3B shows a cross section along the probe axis for $\nu_{\text{probe}} = \nu_{\text{COO}^-}$ and for parallel polarization of pump and probe pulses. The negative part at 1550 cm^{-1} is due to the bleaching and $\nu = 1 \rightarrow 0$ stimulated emission of the COO^- stretch mode on the diagonal. The positive

region centered at 1580 cm^{-1} is the low-probe-frequency tail of the diagonal induced absorption of the Gdm_{LF}^+ mode. The negative region at 1610 cm^{-1} is the negative part of the cross peak between the COO^- stretch mode and the Gdm_{HF}^+ mode.

In order to obtain structural information from the 2D-IR spectra, we model them with an exciton model, which is a well established way of analyzing 2D-IR data. [8] It has been shown before that, in addition to coupling between the two CN_3D_6^+ modes, there is also energy transfer between them,[5] but in DMSO this process is slow enough to be neglected at the delay at which we measured the 2D-IR spectra used for the structural analysis (2 ps). Figs. 2F and G show the calculated 2D-IR spectra, which are in very good agreement with the measured ones, specially considering that the difference 2D-IR spectrum between parallel and perpendicular polarizations is reproduced very well (even though it is not included in the fit). The couplings between the three modes involved in the salt bridge (Gdm_{HF}^+ , Gdm_{LF}^+ and Ac^-), and the angles between their transition-dipole moments were parameters of the fit and are listed in Table I. The coupling between the Gdm_{HF}^+ and Gdm_{LF}^+ modes is 9 cm^{-1} , but a large coupling was expected between these vibrational modes because they share atoms and bonds. Remarkably, the coupling between each of the Gdm_{HF}^+ and Gdm_{LF}^+ modes and the COO^- stretch mode is also large, (10 and 7 cm^{-1} , respectively) even though these vibrational modes neither share atoms nor bonds. This coupling rather originates from salt bridging between the two molecules, an interaction that has both an electrostatic and hydrogen-bond nature. The large couplings between the COO^- and the $\text{Gdm}_{\text{HF},\text{LF}}^+$ modes show that the large frequency shifts observed in the IR spectrum upon salt-bridge formation (Fig. 1A) are mostly due to splitting of the coupled modes rather than to a change

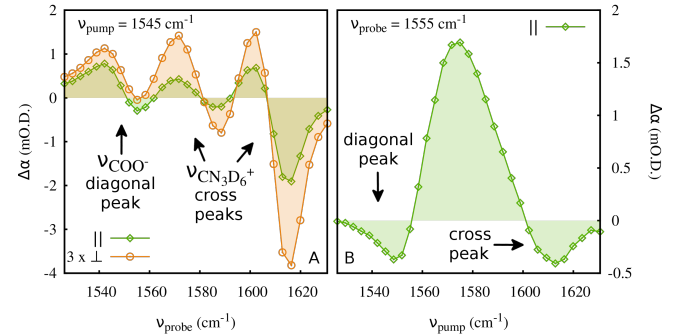


FIG. 3. (A) Cross section along the probe axis of the 2D-IR spectra for parallel and perpendicular polarization of the pump and probe pulses, and for $\nu_{\text{pump}} = \nu_{\text{COO}^-}$. (B) Cross section along the pump axis of the 2D-IR spectrum for parallel polarization of the pump and probe pulses, and for $\nu_{\text{probe}} = \nu_{\text{COO}^-}$.

TABLE I. Coupling β and angle θ between the transition-dipole moments of the COO^- stretch of Ac^- and the two CN_3D_6^+ modes of Gdm^+ as obtained from the fit ($\chi_r^2 = 43$).

Modes	β (cm^{-1})	θ
$\text{Gdm}_{\text{HF}}^+ - \text{Gdm}_{\text{LF}}^+$	9	80°
$\text{Gdm}_{\text{HF}}^+ - \text{Ac}^-$	10	-3°
$\text{Gdm}_{\text{LF}}^+ - \text{Ac}^-$	7	80°

in the local-mode frequencies. The angles between the transition-dipole moments of the salt-bridged vibrational modes are listed in Table I, and these values are in agreement with a planar geometry. The planarity of a salt bridge is often taken as a metric of its quality in X-ray studies, [9] and our results seem to suggest that in DMSO solution, the geometry of an isolated salt bridge, in which steric constraints are absent, is indeed planar. We have performed complementary *ab initio* calculations on the $\text{Gdm}^+ \cdots \text{Ac}^-$ dimer using Gaussian03 [10] at MP2/6-311+G(d) level of theory. The calculation predicts an approximately planar geometry for the salt bridge, in which all three transition-dipole moments lie almost in the same plane, as shown in Fig. 1A. The angle between the transition-dipole vectors of the two Gdm^+ modes is smaller than for isolated guanidinium [5], most likely as a result of salt bridging with the Ac^- ion. We find in the calculation that the Gdm_{HF}^+ mode is antisymmetric with respect to the symmetry axis through the C-C bond of Ac^- , which explains the large coupling with the also antisymmetric COO^- stretch mode. [11] The Gdm_{LF}^+ mode is symmetric with respect to this symmetry axis, so it is remarkable that it also couples strongly to the COO^- stretch mode, despite their different symmetry.

In conclusion, we were able to detect the existence of a salt bridge between Gdm^+ and Ac^- in solution using 2D-IR spectroscopy. We characterize the coupling between two CN_3D_6^+ modes of Gdm^+ and the COO^- stretch mode of Ac^- . We find that the COO^- stretch mode couples more strongly to the high-frequency CN_3D_6^+ mode than

to the low-frequency one, most likely because of their similar symmetry. We determine the geometry of the salt bridge and find that it is in good agreement with a salt-bridge geometry in which the Gdm^+ and COO^- moieties are coplanar.

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